

Oviposition and Isolation of Viable Eggs from *Orius insidiosus* in a Parafilm and Water Substrate: Comparison with Green Beans and Use in Enzyme-Linked Immunosorbent Assay

JEFFREY P. SHAPIRO¹ AND STEPHEN M. FERKOVICH

USDA-ARS CMAVE, 1600/1700 SW 23rd Drive, Gainesville, FL 32608

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ABSTRACT Plant parts are commonly used as oviposition substrates for mass rearing of *Orius* spp., but this system does not permit the isolation of clean intact eggs for use as standards in a yolk protein enzyme-linked immunosorbent assay (ELISA). An oviposition substrate was devised for *O. insidiosus* by forming water-filled domes from Parafilm-M. Eggs were deposited through the Parafilm skin into water where they remained viable for at least 24 h. Eggs were extracted from the domes and collected onto black filter paper and then used to prepare lyophilized yolk protein as a quantitative standard in ELISA tests. In no-choice tests, *O. insidiosus* females oviposited 5.86 eggs per female per d into 'Blue Lake' green beans, *Phaseolus vulgaris* L., and 3.06 eggs into water-filled domes. Hatch rates did not significantly differ, although optimal moisture was critical. In a choice test, *O. insidiosus* females preferred beans to domes (4.02 versus 0.03 eggs per female per 18 h). Females preferred to oviposit into the walls of the major groove of green beans and often oviposited around the lower perimeter of water-filled domes. Lyophilized protein from eggs collected in water-filled domes was successfully and replicably used in the yolk protein ELISA after compensating for reduced immunological activity with a correction factor. Parafilm-covered water-filled domes are therefore useful for collection of eggs for use in the yolk protein ELISA and for other applications that require viable eggs. These domes also may lead to discovery of the phytochemical and physical factors that are responsible for choice of a host plant and its parts for oviposition.

KEY WORDS predator, eggs, oviposition, Parafilm, ELISA

Anthocoridae of the genus *Orius* prey on many small arthropod pests of vegetable and ornamental crops, pests such as thrips, whiteflies, aphids, and mites and on the eggs and small larvae of many Lepidoptera. They are often among the most abundant predators in agricultural habitats and can survive in low-prey situations, successfully developing solely on pollen (Kiman and Yeagan 1985). Although their broad host range and relative ease of rearing now make them useful for augmentative biocontrol worldwide, *Orius* spp. were not mass reared until after 1989 (Schmidt et al. 1995). Mass-rearing methods for *Orius* continue to improve, with decreased cost and increased efficiency of production as primary goals.

Success in mass rearing *Orius insidiosus* (Say) and other predatory Heteroptera has directed attention to their roles as generalist predators and uses in biological control of arthropod pests. However, many species of predatory Heteroptera, anthocorids among them, can be considered omnivores (Coll 1998, Coll

and Guershon 2002), because they live in tritrophic associations with both prey and plant host. One reflection of this association is the necessity to use plant materials as oviposition substrates in rearing systems. Green beans, *Phaseolus vulgaris* L., have commonly been used as oviposition substrates (Richards and Schmidt 1996), but other plant substrates such as soybean, *Glycine max* (L.) Merr., sprouts (Zhou et al. 1991), roots of germinated broad bean (*Vicia faba* L.) seeds (Murai et al. 2001), geranium (*Pelargonium peltatum* L.) leaves (Alauzet et al. 1992), potato (*Solanum tuberosum* L.) sprouts and bean (*P. vulgaris*) stems (Richards and Schmidt 1996), and inflorescence of farmer's friend (also hairy beggar's tick, *Bidens pilosa* L.; Mendes et al. 2005) also have been used.

Use of green beans as oviposition substrates has been critical in our efforts to isolate oogenic nutritional factors from factitious prey of *O. insidiosus* (Ferkovich and Shapiro 2004a) and from a cell line derived from such prey (Ferkovich and Shapiro 2004b). To bioassay reproductive responses to these factors in adult females, we have measured both rate of oviposition into green beans and yolk protein contents of females. The latter is determined with a yolk protein-enzyme-linked immunoassay (YP-ELISA),

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¹ Corresponding author, e-mail: jshapiro@gainesville.usda.ufl.edu.

which required the production of immunogens and quantitative standards from recently oviposited eggs (Shapiro and Ferkovich 2002). Our need for isolated eggs led to the discovery that *O. insidiosus* readily oviposits into Parafilm-encapsulated water-filled domes, from which the eggs are easily collected. Here, we report a comparison between rates of oviposition into water-filled domes and green beans, isolation of eggs from water-filled domes, and the preparation and use of a lyophilized egg extract in the YP-ELISA.

Materials and Methods

Photography. Insect photographs were taken under fiber optic illumination or a with flash using a digital camera (Coolpix 4500, Eastman Kodak, Rochester, NY) mounted above the trinocular tube of a stereomicroscope (SMZ800, Nikon, Lewisville TX).

Water-Filled Dome Fabrication and Egg Extraction. Water-filled domes were constructed as described for diet encapsulation (Greany and Carpenter 1998). A 15- by 9.5-cm piece of Parafilm-M (American National Can, Chicago, IL) was placed on a vacuum-forming device at room temperature, a vacuum was pulled to form wells in the Parafilm, and wells were filled with 0.4–0.6 ml of analytical grade water. A base of plastic-coated freezer paper was sealed to the Parafilm by using a hot iron, resulting in domes of ≈ 13 mm in diameter by 10 mm in height. After 24-h exposure to ovipositing females, the Parafilm domes were sliced open with a scalpel, and the water and eggs were vigorously rinsed with analytical grade water from a wash bottle into a 47-mm filter holder containing a black 55-mm filter paper disk (#551, Schleicher and Schuell, Keene, NH). Interiors of the Parafilm domes were rinsed free of eggs, and water was removed from the filter device by vacuum, leaving the filter paper slightly moist. Eggs on the disks were counted at 12 \times magnification under a dissecting microscope. Black disks were placed onto white filter paper moistened with 600 μ l of analytical grade water in 100- by 15-mm petri dishes, and dishes were placed into a sealed 2-liter glass baking dish and kept for hatching in an incubator at 25°C and 70–80% RH. Hatched nymphs were counted 3 d later (experiment 1).

Green Beans. 'Blue Lake' green beans were purchased at a local supermarket and placed into an aqueous 3% bleach solution for 10 min, rinsed well in deionizer water, and air-dried. After 24-h exposure to ovipositing females, beans were removed from petri dishes, and eggs were counted under a dissecting stereomicroscope. Beans were placed within petri dishes on moist filter paper as described above, and hatched nymphs were counted 3 d later (experiment 1).

Insects. Adult *O. insidiosus* were received weekly in containers of $\approx 1,000$ insects from Syngenta-Bioline Inc. (Oxnard, CA) (experiment 1) and placed in 400-ml jars (≈ 250 adults per jar) with Hydrocapsules (Analytical Research Systems, Gainesville FL), and shredded wax paper as a substrate, or they were reared

from this strain in a colony for fewer than 10 generations before use (experiment 2). Hydrocapsules are polymeric spheres (1–2 mm in diameter) that enclose purified water with blue food coloring added to trace consumption. Insects were fed four times a week with ≈ 125 mg of eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) stored at -80°C . Colony insects only also were provided with ≈ 150 mg of pollen grains (from a local organic food store) before hatching from green beans. To encourage egg retention, beans were removed from jars 2 d (experiment 1) or 1 d (experiment 2) before experimental setup.

Experiment 1: No-Choice Oviposition Test. For each replicate, a 150-mm petri dish was lined with no. 1 filter paper treated with 1 ml of a preservative solution containing methyl paraben (ICN Biomedicals, Aurora, OH) and sorbic acid (Sigma, St. Louis, MO), each at 0.2% (wt:vol) in analytical grade water. Either three Parafilm-covered water-filled domes or one whole green bean, plus 30 mg of *E. kuehniella* eggs and a vial of water, was added to each dish with 15 females and five males received 2 d previously. Dishes were set up in duplicate for each no-choice treatment (domes or bean). The experiment was repeated 10 times, for a total of 20 replicates. Oviposition substrates were collected after a 24-h oviposition period.

Experiment 2: Choice and No-Choice Oviposition Tests. Adult insects 18–20 d from egg stage (≈ 10 d from adult eclosion) were selected from the colony. Insects were sexed on an ice-cold chilling stage, and 15 females and five males were placed together in 150- by 15-mm plastic petri dishes. Each dish served as a single replicate and contained a 125-mm filter disk (no. 1), 10 Hydrocapsules (Analytical Research Systems), 30 mg of *E. kuehniella* eggs, and either one Blue Lake green bean (thoroughly rinsed with distilled water and dried), two Parafilm water-filled domes filled with analytical grade water, or one bean and two domes. Insects were allowed to oviposit for 18 h before collection and counting of eggs from water-filled domes and in beans as described above. Each choice (bean and domes) or no-choice treatment (bean only or domes only) had four replicates.

Yolk Protein Standards and ELISA Response. Eggs were collected from water-filled domes after 2-h oviposition periods, accumulated in 500- μ l microfuge tubes, and stored at -80°C . An accumulated 1,000 frozen eggs were homogenized by adding 250 μ l of 15 mM $\text{NH}_4\text{Ac}/0.05\%$ bovine serum albumin (BSA), pH 7.25, to each of two 1.5-ml microcentrifuge tubes containing 500 eggs and sonicating for 1 min in a cup sonicator at high power. Insoluble material was pelleted at 20,800 $\times g$, and the pellet was reextracted and re-centrifuged. Combined supernatants were brought to 40 ml in the same buffer and filtered through a sterile 0.22- μ m filter and distributed in 200- μ l aliquots (five egg-equivalents per tube). Aliquots were quick-frozen in a -55°C bath, lyophilized overnight to dryness, and stored at -80°C .

Double antiserum sandwich ELISA tests were run as described previously (Shapiro and Ferkovich 2002). Briefly, a Maxisorp microtiter plate from Nalg Nunc

International (Rochester, NY) was coated overnight at 5°C with 200 μ l (5 μ g/ml) of monoclonal antibody 2F3. The plate was blocked with 1% BSA/0.05% Tween 20 in 0.15 M NaCl/50 mM sodium phosphate, pH 7.2.

One hundred microliters of an extract from 50 frozen *O. insidiosus* eggs homogenized in 10 ml or 100 μ l of lyophilizate of five egg-equivalents diluted to 1.0 ml (each 0.5 egg-equivalents/100 μ l) was diluted in two-fold steps through eight dilutions beginning with 0.5 egg-equivalents per well. After a 2-h incubation, 1F3 detecting monoclonal antibody conjugated to horseradish peroxidase was added and then detected with 100 μ l of TMB substrate (Pierce Chemical, Rockford, IL) and read in a microtiter plate reader (Bio-Tek Instruments, Winooski, VT). The dilution series was curve-fitted with a four-parameter fit (KCJr software, Bio-Tek Instruments), and the four variables were recorded. Values for variable C (the EC_{50}) were compared among samples. Variables were fit according to the following equation (Gerlach et al. 1993): $y = (A - D) / ((1 + (x/C)^B) + D)$, where A and D are the maximum response and the baseline, respectively; B is the slope; and C is the concentration of the antigen representing a 50% response, or EC_{50} .

Eggs were collected for 2-h periods in water-filled domes, accumulating 50 eggs per aliquot for individual native standards that were homogenized the day of the assay (1 aliquot per assay), and 1,000 eggs for mass-homogenized, aliquoted, lyophilized standards. For comparison, separate stored aliquots of the two types of standard were repeatedly simultaneously analyzed as serially diluted standards in YP-ELISA. Response of the assay to two batches of the two types of standards was compared by calculating the concentration of each required for a half-maximal response (EC_{50}) and comparing the EC_{50} values as a ratio of native to lyophilized standards, where a higher EC_{50} denotes lower sensitivity of response.

Statistical Analyses. Statistical analyses by unpaired two-tailed *t*-test or analysis of variance (ANOVA) with Tukey's honestly significant difference multiple range test were conducted using Statistica software, release 7 (StatSoft, Tulsa, OK).

Results

Behavioral and Visual Observations. Females laid most of their eggs in loose clusters in green beans along either side of the midrib in the groove of the bean (Fig. 1). Often, clusters of eggs also were laid around and within the cut end of the pedicel. Fewer eggs were laid around irregularities and abrasions on the surfaces of beans. The egg cap (Fig. 1) projected above the surface, affording an exit for the hatchlings. When digital images of individual eggs within beans were digitally outlined and the outlined area of entire eggs was compared with the area exposed above the surface of the bean, a mean of $10.9 \pm 3.1\%$ ($n = 5$) of the egg profile was exposed. When water-filled domes were placed in colonies of mixed sex, females climbed onto the domes and oviposited through the Parafilm (Fig. 2). Females typically placed their front tarsi at

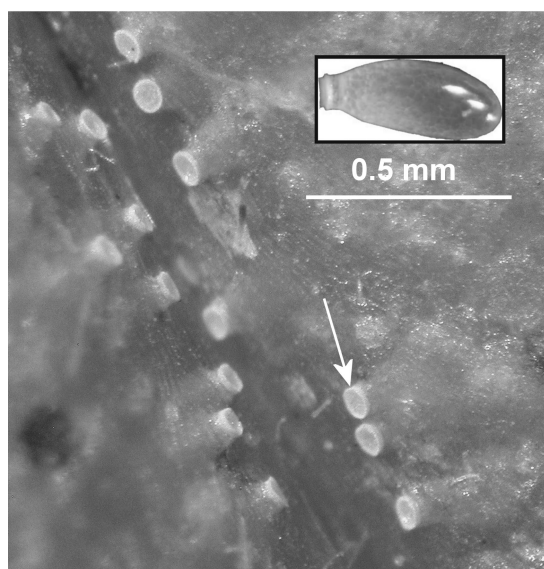


Fig. 1. Eggs of *O. insidiosus* embedded in the surface of a green bean. Top view into groove, which is located diagonally from top left of picture. Arrow indicates an egg cap. Inset, egg isolated from water-filled dome, scaled for direct comparison.

the base of the Parafilm dome to provide leverage for insertion of the ovipositor through the wall of the dome. After oviposition, dissection of water-filled domes revealed that a few eggs remained attached to the interior of the Parafilm skin, but most floated freely in the water within the dome.

Eggs isolated from water-filled domes (Fig. 1, inset) retained their shape and remained viable despite submersion for 0–24 h and subsequent vigorous rinsing from domes onto filter paper. If not kept on a hydrated substrate, eggs quickly dehydrated and shriveled. When isolated eggs were kept on a sparingly hydrated substrate for 3 to 4 d, nymphs hatched and emerged without difficulty. On excessively moistened filter paper, they readily drowned upon hatching.

Experiment 1: No-Choice Oviposition Test. Females that were allowed to oviposit into isolated water-filled domes for 24 h laid 52.5% as many eggs (3.06 ± 1.84 eggs per female, mean \pm SD) as those ovipositing into isolated green beans (5.86 ± 3.18) (Table 1; $F = 11.6$, $P < 0.002$ by one-way ANOVA, $n = 20$). Hatch rates of eggs from domes were 77.3% the rate of those hatching from beans (37.1 ± 18.5 versus $48.1 \pm 14.9\%$ hatch rates); these rates were not significantly different ($F = 3.18$, $P > 0.05$, $n = 20$). Eggs in beans were much more difficult to count than those collected onto black filter paper from domes.

Experiment 2: Choice and No-Choice Oviposition Tests. When females were offered a choice of beans or domes, they overwhelmingly preferred the beans, ovipositing 4.82 eggs per female in beans versus 0.13 eggs per female in domes (Table 2). No eggs were recovered from the domes in three of eight choice replicates, and only one egg was recovered in three other

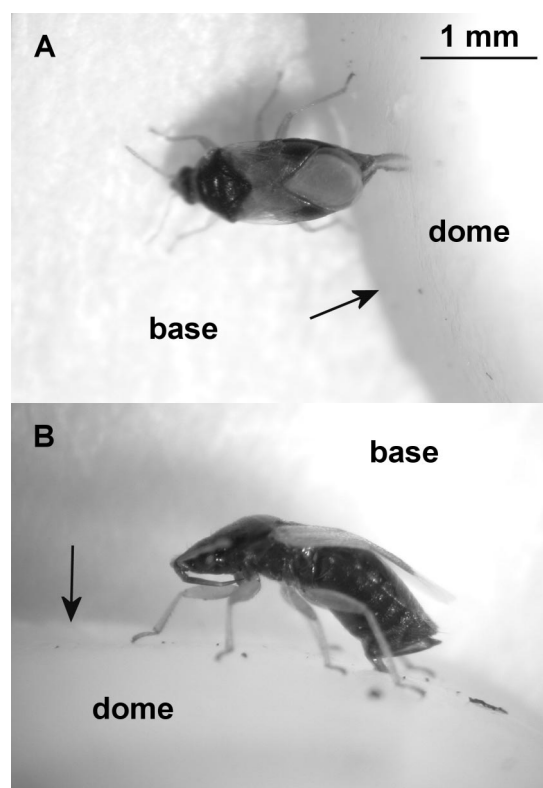


Fig. 2. Females ovipositing into water-filled domes (A) by using base for leverage and (B) on surface of dome. Arrows indicate the interface of base and dome. Scale pertains to A.

replicates. In the no-choice test (beans were absent from dishes with domes and vice versa), females laid significantly more eggs in isolated beans (range 3.9–7.8 eggs per female) than in isolated domes (range 1.9–3.4 eggs per female). Numbers of eggs in this 18-h test were comparable with the 24-h no-choice test in Table 1, although slightly less because of the 18- versus 24-h period (85–90% as many eggs for 18 h compared with 24 h). In contrast to the few eggs recovered from domes in the choice test, however, recoveries from domes in this no-choice test were substantial.

ELISA Response to Eggs or Egg-Lyophilizate. Mass-collected eggs were extracted in a large batch, and soluble protein was collected and aliquoted, quick-frozen, and lyophilized. The immunoreactivities of

Table 1. Oviposition and percentage of hatch of eggs laid over a 24-h period in green beans or Parafilm-covered water-filled domes

Substrate	Eggs/female	% hatch
Bean	5.86 ± 0.71*	48.1 ± 3.3
Water-filled dome	3.06 ± 0.41*	37.1 ± 8.3

Data are presented as mean ± SE ($n = 20$). Asterisk (*) indicates significant difference ($P < 0.002$) by two-tailed t -test. Percentage of hatch did not significantly differ between substrates ($P > 0.05$).

Table 2. Oviposition into water-filled domes and beans during an 18-h period when offered a choice or no choice of the substrate

Substrate	Eggs/female
Choice test	
Bean	4.82 ± 0.53a
Water-filled dome	0.13 ± 0.09b
No-choice test	
Bean	5.27 ± 0.46a
Water-filled dome	2.63 ± 0.23b

Data are presented as mean ± SE ($n = 8$). Means followed by the same letter within a test are not significantly different from those within the same test ($P < 0.0005$; two-tailed t -test).

extracted eggs and equivalent amounts (in egg-equivalents per well) of mass-extracted egg-lyophilizate were determined by ELISA, yielding comparative EC_{50} values (Table 3). The ratio of the EC_{50} of lyophilizate to the EC_{50} of native egg protein was 2.3, demonstrating that the ELISA was 2.3 times as sensitive to protein from homogenized frozen eggs than eggs that had been homogenized, frozen, and lyophilized. Although 56% of the immunoactivity was lost during the process, probably because of freezing, the remaining activity in a batch of aliquots was easily compared with freshly homogenized eggs for expedient and replicable use as ELISA standards.

Discussion

The observations and comparisons of choice with no-choice tests suggest that both physical and phytochemical factors may play roles in oviposition behavior. Bean sutures or the base of water-filled domes were favored for oviposition, suggesting the importance of physical features. Female *O. insidiosus* commonly oviposit into relatively hard plant parts such as stems or fruit, and they may use physical features such as inside angles or surface irregularities to gain leverage for insertion of the ovipositor. For *O. insidiosus*, plants are critical in providing oviposition sites (Coll 1996). Embedding eggs in plant material provides protection from predation or parasitism and from abiotic factors. Because nymphs hatched from isolated eggs as efficiently as from beans, the structural support afforded eggs in a solid or gelatinous substrate is not essential. The hydration afforded by the substrate is

Table 3. Relative response of an ELISA to yolk proteins extracted from isolated eggs and serially diluted for immunoassay

Yolk protein preparation	EC_{50} ^a
Native	36 ± 13
Lyophilized	117 ± 25
Ratio (lyophilized:native)	2.3 ± 0.8

Protein was extracted at time of ELISA from batches of fifty 0–2-h-old frozen eggs (native) or mass prepared from 1,000 eggs as lyophilized aliquots and reconstituted for ELISA (lyophilized). Data are presented as mean ± SE ($n = 4$), each represented by the mean of four to six replicates.

^a Effective concentration in egg milliequivalents resulting in 50% of maximal response in a serial dilution, from equation for four-parameter fit.

necessary, however, because isolated eggs desiccated very rapidly. Excessive hydration was not a problem for eggs: they survived for at least 24 h in the aqueous environment of the water-filled dome. It is a problem for hatching nymphs, which easily drowned in small drops of water.

Physiochemical factors may play a role in the initial choice of substrate. Paraffin wax, Parafilm, and waxes found in plant cutin and suberin on epidermal surfaces all contain long chain hydrocarbons, waxes, wax esters, or a combination (Post-Beittenmiller 1996). In herbivores, these lipids may attract or deter oviposition, because of physical as well as chemical characteristics (Eigenbrode and Espelie 1995). For example, in the diamondback moth, *Plutella xylostella* (L.) (Plutellidae), heavy wax blooms deterred oviposition, yet impressing Parafilm with surface components of canola leaves resulted in increased oviposition by the moth (Justus et al. 2000), indicating a possible chemical effect. The type of waxy surface on leaves of the cabbage *Brassica oleracea* variety *capitata* L. affected the impact of three generalist predators—*Chrysoperla carnea* (Stephens) (Chrysopidae), *O. insidiosus* (Anthocoridae), and *Hippodamia convergens* Guerin-Meneville (Coccinellidae)—on populations of their prey (Eigenbrode et al. 1995). Glossy leaves allowed the strongest effects of predator against prey populations, likely because of physical effects, such as improved mobility and attachment of the predators. This was confirmed in *O. insidiosus*, the adult coccinellid *H. convergens*, and larval lacewing *C. carnea*, and wax crystals in epicuticular wax blooms were implicated as deterrent to movement (Eigenbrode et al. 1996). A comprehensive review, however, found little study, and no clear impact, of the surface wax morphology or chemistry on oviposition preferences of insect carnivores (Eigenbrode 2004). If clear phytochemical or physical effects of epicuticular wax on predators can be shown and specific factors identified, oviposition stimulants can play an important role in the mass rearing of natural enemies (Gregoire et al. 1992). Conceivably, they could be impregnated in the surface, or dissolved in the interior, of mass-produced water-filled domes.

Retrieval of clean intact eggs was also a practical necessity for development and use of a yolk protein ELISA (Shapiro and Ferkovich 2002). Eggs for immunogen and monoclonal antibody production were initially dissected laboriously from fecund females. If oviposited into plant material or into artificial substrates fashioned from stretched Parafilm covering gelatin or carrageenan domes, eggs could not be removed without destroying them, or at least with great difficulty, and hatch rates were poor within the substrate (Castane and Zalom 1994). Parafilm-covered water-filled domes had been routinely used in our laboratory to provide water to *Podisus maculiventris* (Say) and *O. insidiosus*, so a chance observation that *O. insidiosus* oviposited into those domes afforded the opportunity to improve on the substrate.

Our results demonstrated that Parafilm-covered water-filled domes serve as good oviposition substrates in

no-choice situations. However, when a choice was offered between the water-filled domes and green beans, fewer than 3% as many eggs were laid in domes as in beans. Three of eight replicates contained no eggs, and four of eight contained only one or two eggs. Thus, beans are highly favored over the water-filled domes when a choice is available. Although one-half as many eggs were laid into water-filled domes as into green beans in no-choice tests, the numbers of eggs laid in water-filled domes (3.06 and 2.63 eggs per female per 24 h in Tables 1 and 2, respectively) were comparable with those observed by other authors in continuous oviposition tests. For example, Kiman and Yeargan (1985) observed 1–3.2 eggs per female per d, and Castane and Zalom (1994) recorded 2.8 and 2.9 eggs per female per d on gelatin and bean substrates, respectively. The high numbers that we observed were probably enhanced by withholding any substrate for 24 h before the 24-h oviposition period. Additionally, rapid declines in oviposition rate begin to occur ≈ 10 d after emergence, and these are not factored into our 24-h oviposition tests.

The water-filled domes thus served well as egg collection devices, although they lack attributes necessary for use as one-step substrates in mass rearing. They are impenetrable and unsurvivable by hatching nymphs. However, eggs survive submersion for at least 24 h, and reasonable hatch rates (37.1 versus 48.1% from beans, an insignificant difference) can be assured by extracting eggs from the water-filled domes and maintaining them in a moist, not water-saturated, environment. This rate of hatch for isolated eggs rivals the rates observed for eggs in many plant-derived substrates such as potato sprouts and bean stems and pods (Richards and Schmidt 1996); a Parafilm substrate resulted in no hatch in the same study. Microbial contamination, a problem noted by numerous workers (Castane and Zalom 1994, Schmidt et al. 1995, Murai et al. 2001) can be minimized using domes. The non-toxic preservatives methyl paraben and sorbic acid must be used in moist petri dishes to prevent fungal growth. These chemicals apparently had little or no detrimental effect on survival and hatch rates.

A replicable, precise and accurate ELISA requires stringent control of quantitative standards. Standards that are durable and require no further preparation at the time of assay will expedite the procedure. Water-filled domes proved invaluable for mass collection of clean eggs with which to prepare yolk protein standards.

Water-filled dome substrates also have potential in other applications, whenever eggs of anthocorids need to be studied in an isolated system. For example, water-filled domes have an advantage over a carrageenan substrate that was used previously to study the time course of yolk protein disappearance in developing eggs (Shapiro and Ferkovich 2002). We envision other applications: studying biochemical changes during egg and embryonic development; behavior of newly hatched nymphs; egg structure, function, and variability; and physiological responses of eggs to various physical and simulated host plant environments.

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References Cited

- Alauzet, C., D. Dargagnon, and M. Hatte. 1992. Production of the heteropteran predator: *Orius majusculus* (Het.: Anthocoridae). *Entomophaga* 37: 249–252.
- Castane, C., and F. G. Zalom. 1994. Artificial oviposition substrate for rearing *Orius insidiosus* (Hemiptera: Anthocoridae). *Biol. Control* 4: 88–91.
- Coll, M. 1996. Feeding and ovipositing on plants by an omnivorous insect predator. *Oecologia* (Berl.) 105: 214–220.
- Coll, M. 1998. Living and feeding on plants in predatory Heteroptera, pp. 89–129. In M. Coll and J. R. Ruberson [eds.], *Predatory Heteroptera: their ecology and use in biological control*. Entomological Society of America, Lanham, MD.
- Coll, M., and M. Guershon. 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annu. Rev. Entomol.* 47: 267–297.
- Eigenbrode, S. D. 2004. The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. *Arthropod Struct. Dev.* 33: 91–102.
- Eigenbrode, S. D., and K. E. Espelie. 1995. Effects of plant epicuticular lipids on insect herbivores. *Annu. Rev. Entomol.* 40: 171–194.
- Eigenbrode, S. D., S. Moodie, and T. Castagnola. 1995. Predators mediate host plant resistance to a phytophagous pest in cabbage with glossy leaf wax. *Entomol. Exp. Appl.* 77: 335–342.
- Eigenbrode, S. D., T. Castagnola, M. B. Roux, and L. Steljes. 1996. Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. *Entomol. Exp. Appl.* 81: 335–343.
- Ferkovich, S. M., and J. P. Shapiro. 2004a. Comparison of prey-derived and non-insect supplements on egg-laying of *Orius insidiosus* maintained on artificial diet as adults. *Biol. Control*. 31: 57–64.
- Ferkovich, S. M., and J. P. Shapiro. 2004b. Increased egg-laying in *Orius insidiosus* (Hemiptera: Anthocoridae) fed artificial diet supplemented with an embryonic cell line. *Biol. Control*. 31: 11–15.
- Gerlach, R. W., R. J. White, S. N. Deming, J. A. Pelasota, and J. M. Vanemon. 1993. An evaluation of five commercial immunoassay data analysis software systems. *Analyt. Biochem.* 212: 185–193.
- Greany, P. D., and J. E. Carpenter. 1998. Culture medium for parasitic and predaceous insects. U.S. Patent 5,799,607.
- Gregoire, J. C., D. Couillien, A. Drumont, H. Meyer, and W. Francke. 1992. Semiochemicals and the management of *Rhizophagus grandis* Gyll (Col, Rhizophagidae) for the biocontrol of *Dendroctonus micans* Kug (Col, Scolytidae). *J. Appl. Entomol.* 114: 110–112.
- Justus, K. A., L. M. Dossall, and B. K. Mitchell. 2000. Oviposition by *Plutella xylostella* (Lepidoptera: Plutellidae) and effects of phylloplane waxiness. *J. Econ. Entomol.* 93: 1152–1159.
- Kiman, Z. B., and K. V. Yeargan. 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. *Ann. Entomol. Soc. Am.* 78: 464–467.
- Mendes, S. M., V.H.P. Bueno, and L. M. Carvalho. 2005. Suitability of different substrates for oviposition of the predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *Neotrop. Entomol.* 34: 415–421.
- Murai, T., Y. Narai, and N. Sugiura. 2001. Utilization of germinated broad bean seeds as an oviposition substrate in mass rearing of the predatory bug, *Orius sauteri* (Poppius) (Heteroptera: Anthocoridae). *Appl. Entomol. Zool.* 36: 489–494.
- Post-Beittenmiller, D. 1996. Biochemistry and molecular biology of wax production in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 405–430.
- Richards, P. C., and J. M. Schmidt. 1996. The suitability of some natural and artificial substrates as oviposition sites for the insidious flower bug, *Orius insidiosus*. *Entomol. Exp. Appl.* 80: 325–333.
- Schmidt, J. M., P. C. Richards, H. Nadel, and G. Ferguson. 1995. A rearing method for the production of large numbers of the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera, Anthocoridae). *Can. Entomol.* 127: 445–447.
- Shapiro, J. P., and S. M. Ferkovich. 2002. Yolk protein immunoassays (YP-ELISA) to assess diet and reproductive quality of mass-reared *Orius insidiosus* (Heteroptera: Anthocoridae). *J. Econ. Entomol.* 95: 927–935.
- Zhou, W. R., R. Wang, and S. B. Qiu. 1991. Use of soybean sprouts as the oviposition material in mass rearing of *Orius sauteri* (Het.: Anthocoridae). *Chin. J. Biol. Control* 7: 7–9.

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